

## **Pycnogenol® supplementation and its beneficial effects in healthy rats**

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**P**ycnogenol® is a procyanidin-rich extract from French maritime pine. It is reported that it has as an antioxidant effect in a variety of physiological conditions as the other proanthocyanidine resveratrol. It is 50 times more potent than vitamin E and 20 times more than vitamin C. Pycnogenol® has strong antioxidant profile as shown by experimentally in vivo and in vitro studies; however, it has to be confirmed in clinical trials.<sup>1,2</sup> Being water-soluble, Pycnogenol® is a water soluble substance, therefore, it works in the liquid portions inside the cell and intercellular spaces. Pycnogenol® is especially good at neutralizing the hydroxyl radical, the superoxide radical, singlet oxygen, and the potential oxidant peroxyxynitrate. Hence, it has found to be reducing the risk of cancer, strengthening the immune system, prevention of premature aging and degenerative diseases. In vivo supplementation of Pycnogenol® to aged animals results in biological and behavioral changes. Pycnogenol® supplementation to humans resulted in significantly reduced serum low density lipoprotein and increased plasma oxygen radical absorbance capacity.<sup>2,3</sup>

Hence, the aim of this study was to investigate the effects of Pycnogenol® on blood glucose, systolic arterial blood pressure, oxidant/antioxidant parameters also their cofactors as zinc and copper concentrations in healthy rats.

Control group was constituted of 12 male Wistar albino rats, 10-12 weeks of age, weighing approximately 284g. For experimental group, 12 male rats, 10-12 weeks of age, weighing 287g were used. The rats were housed in individual cages in a climate-controlled room with an ambient temperature of  $22\pm 1^{\circ}\text{C}$  for 6 weeks and were in a light regulated space for approximately 12 hours day and night cycles and at a relative humidity of  $60\pm 10\%$  and given a standard laboratory diet and water before experimental procedure.

To have measurable significant effects, Pycnogenol® was recommended as 30 mg/kg/day for administration orally. Thus, in this study, 30mg/kg/day Pycnogenol® was administered in drinking water during 6 weeks in experimental group and to achieve the daily dose intake completely, the volume of water drunk by each rat was measured daily. At the beginning and at the

end of the study, after one week training period, blood pressures and heart rates were recorded by tail-cuff pletysmography both in control and in experimental groups. Each day conscious rats was placed in their maintenance cages at  $28^{\circ}\text{C}$  for 2 hours. Before arterial blood pressure measurements rats were taken to a heated cage (maximum  $32^{\circ}\text{C}$ ) and allowed to rest inside the cage for 15 minutes. For measurement, rat tails were placed inside a tail cuff, and the cuff was inflated and released several times to allow conditioning of the animals to the procedure. A minimum of 4 consecutive measurements was taken and was recorded by a computer. The heart rates were also measured and recorded. During the study, the changes in body weight were evaluated and blood glucose was determined by glucometer. For the other measurements, blood samples of control and experimental groups were drawn and put into the heparinized tubes. The plasma kept in polypropylene tubes, and each sample was studied rapidly. Red cell copper-zinc superoxide dismutase (Cu-Zn/SOD), catalase activities, plasma malondialdehyde (MDA) concentrations, and plasma and red cell copper and zinc concentrations were measured. Superoxide dismutase, catalase activities and MDA concentrations were determined spectrophotometrically. Plasma and red cell zinc and copper concentrations were determined by atomic absorption spectrophotometry, according to Perkin-Elmer's principles. Both zinc and copper are pro- and anti-oxidant trace elements, which provide stability and activity of anti-oxidant enzymes studied in this research.<sup>1</sup> All chemicals used were provided from Germany (MERCK) and pieces of equipment were supplied from Turkey (tail-cuff pletysmography) and USA (atomic absorption spectrophotometer and dual-beam spectrophotometer).

The study was performed between March and June 2009 at the Pathophysiology Laboratories of Ankara University. Researchers followed the Declaration of Helsinki and the NIH Guiding Principles in the Care and Use of Animals. The study was approved by the Ethics Committee of Ankara University, Ankara, Turkey.

All data are expressed as median (minimum-maximum). Shapiro-Wilk test was used for normality testing. Mann-Whitney U test was used to evaluate the difference between 2 groups in terms of non-normally distributed continuous variables. Differences within groups were assessed by Wilcoxon-Signed Ranks test. *P*-values less than 0.05 were considered significant. SPSS for Windows 11.5 was used for statistical analysis

Results showed that there were significant decreases in both body weight gain (control group: 279 g in first

**Table 1** - The results of control and experimental groups.

Variables	Control		Experimental group		P-value
	Median	Minimum-maximum	Median	Minimum-maximum	
Blood glucose (mg/dL)	209	189-300	156.5	116-178	<0.001*
Systolic arterial blood pressures (mm Hg)	165	141-280	139.5	114-156	<0.001*
Heart rates (beat/min)	373	340-389	360	334-384	0.043*
Cu-Zn/SOD (U/g Hb)	4494.5	4000-5128	5263	5000-5555	<0.001*
Catalase (k/g Hb)	158.4	156-159.1	167.2	161-176	<0.001*
MDA (nmol/mL)	10,54	8,5-11.15	4.3	1.2- 6.4	<0.001*
Plasma Zn (µg/dL)	129	109.3-156	93.5	80-110	<0.001*
Plasma Cu (µg/dL)	100	90-120	117	90-146	0.030*
Red cell Zn (µg/mL)	6.75	6,3-7.4	8.17	7.45-9.48	<0.001*
Red cell Cu (µg/mL)	0.30	0.22-0.34	0.30	0.22-0.37	0.662

All data are expressed as median (minimum-maximum). \*statistically significant  
Cu-Zn/SOD - copper-zinc superoxide dismutase, catalase activities, plasma MDA - malondialdehyde

day and 340 g in last day [ $p<0.002$ ] and experimental group 290 g in first day and 285.5 g in last day [ $p=0,304$ ] and blood glucose concentrations in experimental group compared to control group. Pycnogenol® administration decreased also the blood pressures of rats significantly. There were significant decreases in plasma MDA and plasma zinc concentration values in experimental group. On the other hand, Cu-Zn/SOD, catalase activities and plasma copper and red cell zinc concentrations were found to be increased significantly after Pycnogenol® administration. The results are shown in Table 1.

Proanthocyanidins are large class of polyphenols, like natural phenols such as Pycnogenol® and resveratrol; are potent antioxidants. They scavenge free radicals, allowing cells to regenerate in state of degenerate. Pycnogenol® contains 65-75% proanthocyanidins. The studies about the Pycnogenol® effects in various disease conditions were shown beneficial, but its safety and effectiveness are still had to be proven with human studies<sup>1,2</sup>. In this study, after Pycnogenol® administration, blood glucose levels were decreased significantly when compared to controls ( $p<0,001$ ). In some reports, it was proposed that Pycnogenol® has lowering effect on fasting and postprandial blood glucose levels. Pycnogenol® that containing catechins and epicatechins improve weight maintenance, through thermogenesis, fat oxidization, and sparing fat free mass. Hence, in Pycnogenol® treated group there were also significant decreases in body weight gains ( $p<0,001$ ) supporting the metabolic effects of the agent.<sup>3,4</sup> It was found in this study that systolic arterial blood pressure was significantly ( $p<0,001$ ), but heart rate was slightly decreased ( $p=0,043$ ) in Pycnogenol® treated group. In a study, also supplementation with Pycnogenol® over a period of 8 weeks significantly reduced systolic

blood pressure; however, diastolic component was not changed; this result related with its vasodilatory effect through nitric oxide (NO) release by endothelial cells.<sup>3,5</sup>

Pycnogenol® has been shown to scavenge hydroxyl radical, hydrogen peroxide, superoxide anion nitric oxide radicals. In our study, significant increases in Cu-Zn/SOD and catalase activities in experimental group were detected ( $p<0,001$ ,  $p<0,001$ ). In one study, the activities of endogenous antioxidant enzymes (SOD, CAT, GSH-Px, GSSG-R) in streptozotocin-induced diabetic rats were significantly increased following Pycnogenol® administration. These changes were also associated with a significant decrease in blood glucose levels in diabetic rats. In one study, it was reported that, Pycnogenol® suppressed peroxide generation as showing an enhancing effect on free radical scavenging activity of superoxide dismutase. In the same study, Pycnogenol® was reported to have enhancing effect on catalase, Cu-Zn/SOD also glutathion-peroxidase. Additionally, it was shown that Pycnogenol® increases life span of ascorbate radical and helps in regeneration of vitamin E. It was also demonstrated that throughout the Pycnogenol® supplementation period of 3 weeks in human, a significant increase of oxygen radical absorbance capacity in plasma was determined. Pycnogenol® in addition to its antioxidant action, can also protect from damage caused by lipid peroxidation by-products such as MDA. By increasing Pycnogenol® concentrations, the amount of MDA and/or MDA such as products (TBARP) formed by the lipid peroxidation were significantly inhibited. These effects come from chemical action of Pycnogenol® as well as its ability to interact directly with cell membranes and/or penetrate the membrane. Thus, this process induces modification of the lipid bilayer and lipid-protein interactions. Consistently with these data,

MDA concentration in our study, was also found to be decreased ( $p < 0,001$ ).<sup>1,2</sup> Hence, serum and red cell zinc and copper concentrations, being cofactors of Cu-Zn/SOD, were also measured in this study. In Pycnogenol® treated group plasma zinc concentration was decreased and red cell zinc concentration was increased significantly ( $p < 0,001$ ,  $p < 0,001$ ). The increase in red cell zinc concentration might be due to elevated zinc need for increased Cu-Zn/SOD activity. Zinc has been shown also to antagonize the catalytic properties of the redox-active transition metals, such as copper, and Pycnogenol® regarding its contribution to antioxidant defense mechanism, might affect to increase intracellular zinc concentration. In our study, plasma copper concentration was increased slightly while red cell copper concentration did not change. It was not surprising to find increased plasma copper concentration while there was a decrease in plasma zinc concentration, but it was difficult to interpret the values of red cell copper.<sup>1</sup>

In conclusion, Pycnogenol® by decreasing blood pressure, reducing blood sugar levels, inhibiting oxidative stress and enhancing antioxidant enzyme activities shows beneficial effects on metabolic and cardiovascular health. In future research, with these beneficial effects, Pycnogenol® supplementation might be useful in the approach to the treatment of metabolic and cardiovascular diseases.

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